Response to Sandford et al.: *PRICKLE2* Variants in Epilepsy: A Call for Precision Medicine

To the Editor: With the arrival of massively parallel sequencing (MPS), we now face the situation where we often discover several rare mutations in a single individual. So, how do we know which genes are associated with which phenotypes? Although this issue is common in 2016, it was not always the case. In the not-so-distant past, gene sequencing itself was a laborious process, and many genetic studies necessarily took a candidate-gene approach. The letter from Sandford et al. struggles with this recent history.[1]

Sandford et al. write that two siblings with epilepsy and a variant in *PRICKLE2* (MIM: 608501), reported by Tao et. al.,[2] also harbor *POLG* (MIM: 174763) mutations—an important clinical result for this family. As they point out, *POLG* mutations were known to be involved with epilepsy and ataxia as early as 2001— some 10 years before our report,[2] which Dr. Bird also co-authored. They also note that the sibling relationship was described by Bird and Shaw in 1978.[3] So, given that Dr. Bird had the DNA since 1978 and that *POLG* mutations were described in 2001, why was DNA submitted for *PRICKLE2* variant analysis with us in 2011, and why is he reporting on *POLG* mutations in 2016? The answer, in part, is that genotyping individuals was not always as easy as it is today.

The answer could also be that *PRICKLE2* variants might indeed be part of this family's phenotype. Certainly, most of the family's phenotype (e.g., the brain malformation) corresponds to that of other individuals with *POLG* mutations, but seizures and the degree of seizure severity are variable among those with *POLG* mutations. Could the *PRICKLE2* variants in these two siblings contribute to the seizure phenotype?

Recently, results by others have strongly suggested that *PRICKLE2* variants are associated with epilepsy. For example, a recent report described whole-exome sequencing in an individual with epilepsy, intellectual disability, and anxiety; this person harbors a de novo frameshift variant (c.380delG [p.Glu127fs*46] [GenBank: NM_198859.3], not found in the Exome Aggregation Consortium [ExAC] Browser) in *PRICKLE2*.[4,5] Interestingly, this individual also has one other de novo variant—a single-nucleotide variant (c.5614A>G [p.Met1872Val] [GenBank: NM_004380.2]) in *CREBBP* (MIM: 600140)—and children with *CREBBP* mutations have been reported to have Rubinstein-Taybi syndrome 1 (MIM 180849), although this individual was not reported to have Rubinstein-Taybi syndrome 1. Moreover, some but not all individuals with *CREBBP* mutations have epilepsy. So, is the de novo *PRICKLE2* frameshift mutation the sole cause of this individual's epilepsy, is the *CREBBP* mutation involved, or does the combination of the two lead to the condition? We cannot answer these questions with DNA sequencing alone.

Similarly, we recently described a de novo mutation (not in the ExAC Browser) in the closely related gene *PRICKLE1* (MIM: 608500) in a fetus with polymicrogyria (comorbid with epilepsy in most affected individuals).[6] The previously reported epileptic individuals with *PRICKLE1* variants had inherited their *PRICKLE1* alleles and did not have polymicrogyria.[7] This de novo mutation represents a more severe case than those previously described. In retrospect, were some individuals mistakenly diagnosed with other disorders when their brain malformations and/or epilepsy were in fact caused by *PRICKLE1* and/or *PRICKLE2* mutations? This will most likely be answered in the near future, as clinical MPS data become widely available.

Sandford et al. state, "Other papers have confirmed that large heterozygous deletions, including *PRICKLE2*, on chromosome 3 are associated with autism, but nothing about epilepsy was mentioned in this or other publications." This is simply false. As early as 1981, in a child with an interstitial deletion that would encompass *PRICKLE2* (although banding was low resolution at that date) "grand mal seizures appeared at 6 months of age but were successfully treated."[8] Even more definitively, in 1997 a publication described a de novo interstitial deletion that encompassed *PRICKLE2* in a baby in whom "seizures started on the first day of life and were treated with phenobarbital."[9] This same child is noted as having seizures in another 2014 publication that specifically diagramed *PRICKLE2* in the deletion.[10] Thus, there are reports of *PRICKLE2* deletions in children with epilepsy in other publications.

Sandford et al. also point out that one of the originally described *PRICKLE2* variants (c.1813G>T [p.Val605Phe] [GenBank: NM_198859.3]) is seen in 2 of over 60,000 individuals represented in the ExAC Browser, but this certainly does not definitively explain the functional role of any allele. The ExAC Browser includes individuals with neurodevelopmental disorders; moreover, with a minor allele frequency of 1.65×10^{-5}, the allele is quite rare. In addition, this same *PRICKLE2* missense variant (but no other disease-implicated variants) was described upon exome sequencing of a 2-year-old with multiple medical issues, including developmental regression and episodes concerning seizures.[4]

Sandford et al. further claim that the functional tests in Tao et al.[2] are "not consistent," even though they showed seizure susceptibility in humans, mice, and fruit flies with mutations in *PRICKLE2* orthologs. Functional biology has many variables and can have a wide range of results. Nevertheless, the finding of more than one rare mutation in a

given individual points to the urgent need to perform functional, personalized, precision-medicine experiments in the age of MPS. Newly described variants must be functionally validated, as rigorously as possible, in an effort to make definitive diagnoses and find new treatments. In the case of *PRICKLE2*, we already published electrophysiological data relevant to specific subject variants in animal and neuronal cell models,[11] and we and others are now poised to use gene editing (using CRISPR/Cas9 in subject-derived immunocompetent stem cells) to correct individual variants and assess their pathogenecity.[12]

In conclusion, our original paper[2] and a brief literature review implicate *PRICKLE2* mutations in epilepsy. We propose that the letter (from Dr. Bird and colleagues) in response to our original 2011 manuscript (from Dr. Bird and us) entitled "Mutations in Prickle Orthologs Cause Seizures in Flies, Mice, and Humans" could be a few-sentence corrigendum better titled "One Family with a Rare *PRICKLE2* Mutation, Epilepsy, and a Brain Disorder Also Has *POLG* Mutations that in Retrospect Explain Their Extreme Phenotype."

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Web Resources

The URLs for data presented herein are as follows:

ExAC Browser, http://exac.broadinstitute.org
OMIM, http://www.omim.org/
RefSeq, http://www.ncbi.nlm.nih.gov/refseq/

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